PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

SAVERIO C. FALCO
SHARON J. KEELER
JANET A. RICE

CASE NO.: BB-1037-D

APPLN. NO.: 08/823,771

GROUP ART UNIT: 1638

FILED: MARCH 24, 1997

EXAMINER: E. MCELWAIN

FOR: CHIMERIC GENES AND METHODS FOR INCREASING THE LYSINE AND THREONINE CONTENT OF THE SEEDS OF PLANTS

Date: FEBRUARY 16, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

Declaration of Dr. Carl Falco Pursuant to 37 CFR §1.132

I, Saverio Carl Falco, am a citizen of the United States of America, residing at 1902 Millers Road, Arden, Delaware 19810, United States of America, and I declare as follows:

1. I am one of the above-identified inventors named in this application. I am a graduate of Rutgers University of New Brunswick, New Jersey with a B.A. degree granted in 1971 with high honors and distinction in physics. I received a Ph.D. in 1977 from the University of Chicago in biochemistry and molecular biology. From 1977 to 1981 I was a National Institutes of Health postdoctoral fellow at the Massachusetts Institute of Technology. I have been employed by E. I. du Pont de Nemours and Company since 1981 directing and conducting research in plant genetic engineering.

2. I have reviewed the Office Action dated November 22, 2000. I am aware that this declaration is being submitted to address the concerns set forth on page 3 of the Office Action that the "Declaration of Falco teaches use of a bifunctional LKR/SDH gene to identify mutants produced by transposon mutagenesis. This plant does not contain a foreign LKR gene. In addition, the Declaration of Falco teaches of a combination DHDPS gene without an AK gene. Thus, the Declaration of Falco

does not teach a plant with a foreign LKR gene and a foreign DHDPS gene . . . it remains unpredictable what the results would be of introducing just the LKR gene and the DHDPS gene into a plant."

3. It was stated in paragraph 10 of my declaration previously submitted on August 24, 2000 that a co-transformation experiment in which a chimeric gene designed for co-suppression of LKR was combined with a chimeric gene for expression of lysine insensitive DHDPS was in progress. That experiment was expected to yield transformants that produced seeds with higher free lysine levels than transformants from a parallel experiment using the DHDPS gene alone. The results of those experiments have now been obtained and they do confirm the prediction that transformants comprising the chimeric gene designed for co-suppression of LKR and the chimeric gene for expression of lysine insensitive DHDPS produced seeds with higher free lysine levels than transformants from a parallel experiment using the DHDPS gene alone. These results are depicted in Figure 2 and Table 1.

4. The chimeric genes used for the experiments were:

i) corn globulin1 promoter/corn chloroplast transit sequence/ Corynebacterium dapA gene/corn globulin1 3'UTR; and

ii) corn 27kd zein promoter/fragment of corn LKR-SDH cDNA/corn 10kd zein 3' UTR

Seeds from many transformation events from each experiment were analyzed for free lysine content. It is clear from the data presented in Figure 2 that the best seeds obtained from the co-transformation experiment had considerably higher free lysine levels than the best seeds obtained from the transformation experiment where only the DHDPS gene was used. The average free lysine level from the 30 highest lysine seeds, or from the 70 highest lysine seeds, was about 2-fold higher for the co-transformation experiments compared the DHDPS only experiment.

5. It also was stated in paragraph 10 of my previous declaration submitted on August 24, 2000 that an LKR co-suppression transformant which showed an increased seed free lysine phenotype for several generations, and behaved genetically as a single locus transgene insertion, was crossed to a transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. Results from that experiment, which were not available at the time of the previous declaration, have confirmed the expectations expressed there, namely that seeds carrying both transgene loci will have higher free lysine levels than either parent. The data are presented in Figure 1.

6. In this experiment described in paragraph 5 above, transgenic lines homozygous for an insertion of DHDPS and AK genes, or homozygous for the co-suppressing LKR/SDH gene, were each crossed to a wild type corn line or to each

other. The F1 progeny seed from these crosses are hemizygous for the DHDPS and AK transgenic insertion, the co-suppressing LKR/SDH transgenic insertion, or both. Each cross was repeated at least 5 times, and seeds from the resulting corn ears were harvested and analyzed for free lysine levels. The results depicted in Figure 1 are averages derived from these repetitions. These results show the dramatic increase in free lysine resulting from the combination of increasing the synthesis of lysine via expression of the DHDPS gene and blocking the major pathway for lysine catabolism by co-suppressing the LKR/SDH gene.

7. Parenthetically, it is noted that a concern was raised in the Office Action dated November 22, 2000 that results from combining the DHDPS and AK transgenic insertions with a co-suppressing LKR/SDH transgenic insertion would not be predictive of combining a DHDPS only transgenic insertion with a co-suppressing LKR/SDH transgenic insertion. It is noted that there is evidence in the subject application that AK plays a secondary role to DHDPS for increasing the synthesis of lysine.

For example, it was demonstrated for (i) rapeseed transformants on page 31 at lines 18 – 24 of the specification that :

"Transformants expressing DHDPS protein showed a greater than 100-fold increase in free lysine level in their seeds. There was a good correlation between transformants expressing higher levels of DHDPS protein and those having higher levels of free lysine. One transformant that expressed AKIII-M4 in the absence of *Corynebacteria* DHDPS showed a 5-fold increase in the level of free threonine in the seeds. Concomitant expression of both enzymes resulted in accumulation of high levels of free lysine, but not threonine."

And for (ii) corn transformants (page 33 at lines 15 – 24:

"Free lysine levels in the seeds is increased from about 1.4% of free amino acids in control seeds to 15-27% in seeds of transformants expressing *Corynebacterium* DHDPS alone from the globulin 1 promoter. The increased free lysine was localized to the embryo in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter.

The large increases in free lysine result in significant increases in the total seed lysine content. Total lysine levels can be increased at least 130% in seeds expressing *Corynebacterium* DHDPS from the globulin 1 promoter. . . . Greater increases in free lysine levels can be achieved by expressing *E. coli* AKIII-M4 protein from the globulin 1 promoter in concert with *Corynebacterium* DHDPS."

8. Thus, the gene encoding lysine insensitive AK can enhance the effect of the DHDPS gene on lysine synthesis by increasing overall flux through the biosynthetic pathway. However, AK does not increase lysine when expressed without DHDPS. It is the DHDPS gene that is necessary for increasing the synthesis of lysine. The presence of the AK gene along with the DHDPS gene in the cross described above is inconsequential with respect to proof of the concept that the **combination** of increasing lysine synthesis (which can be achieved using the DHDPS gene alone or in combination with the AK gene) and blocking lysine catabolism (which can be achieved by blocking expression of the LKR/SDH gene via co-suppression) works better than either alone.

9. The genetic cross experiment and the co-transformation experiment described above, taken together with the detailed description of the invention provided in the patent application and the previous declaration, clearly demonstrate that an increased lysine content is achieved when a lysine insensitive DHDPS gene (with or without a lysine insensitive AK gene) is combined with a co-suppressing LKR gene.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Saverio Carl Falco

Feb 16, 2001

Date

Figure 1: Compare DHDPS + AK, csLKR, DHDPS + AK + csLKR

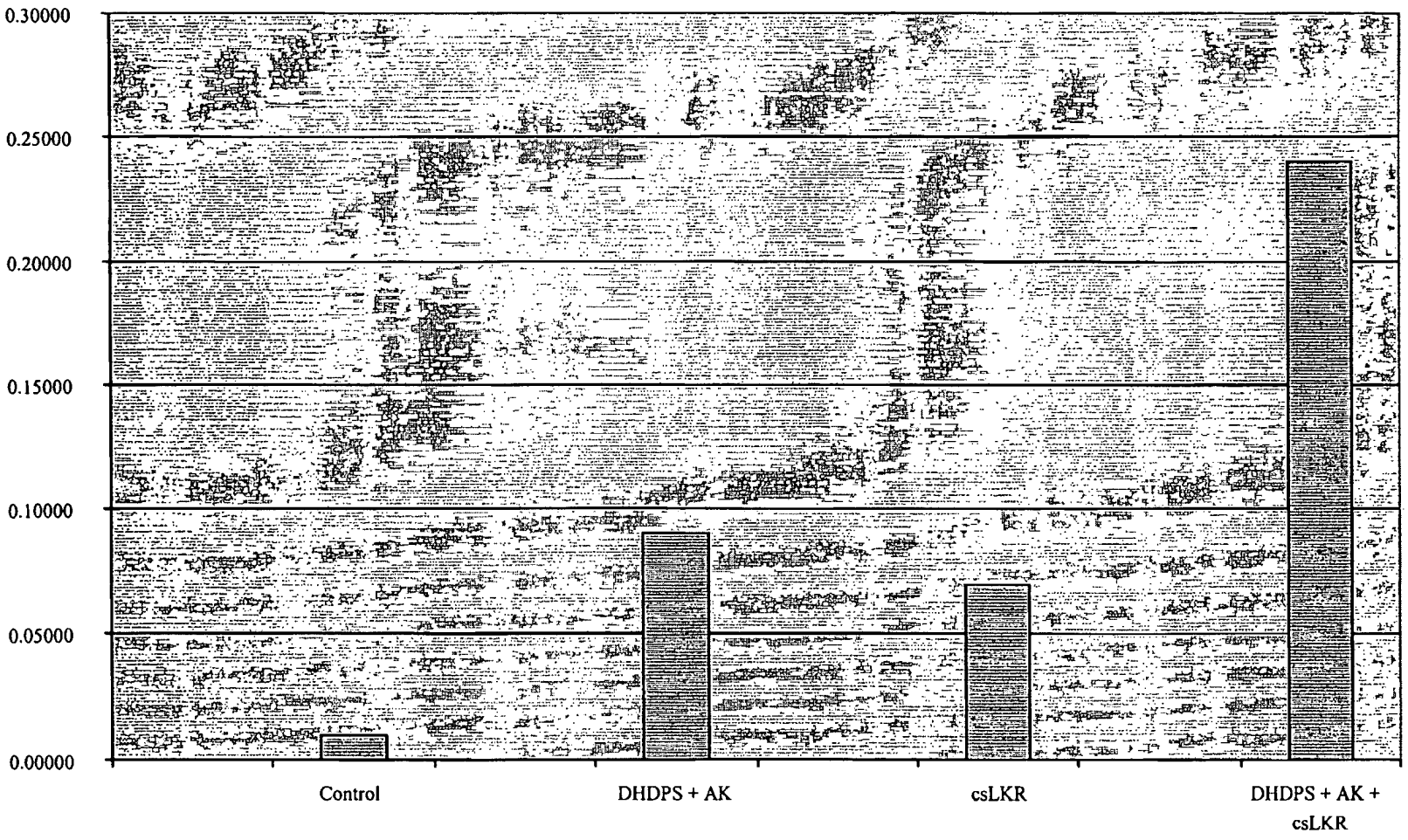

Figure 2: Comparison of seeds from transformation of DHDPS alone vs DHDPS + csLKR

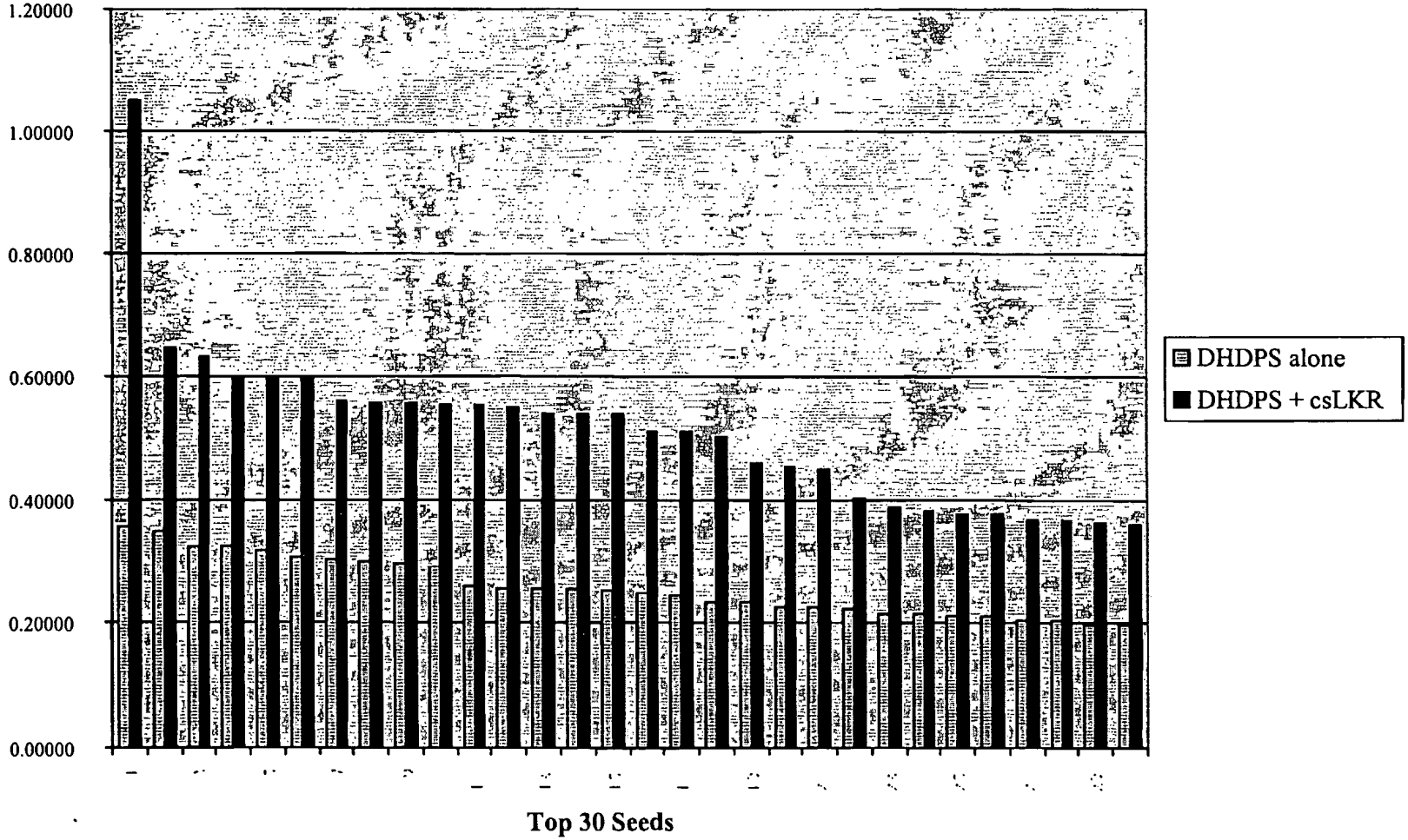

Table 1

	DHDPS alone wt % Free Lys	DHDPS + csLKR wt % Free Lys	wild type corn wt % Free Lys
Avg of best 30 seeds	**0.26**	**0.51**	**0.01**
Avg of best 70 seeds	**0.20**	**0.39**	**0.01**